PTO 02-2099 European Patent No. 0 491 289 A1

DOUBLE-CONFOCAL SCANNING MICROSCOPE

Dr. Stefan Hell

UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, D.C. APRIL 2002
TRANSLATED BY THE RALPH MCELROY TRANSLATION COMPANY

EUROPEAN PATENT OFFICE
EUROPEAN PATENT APPLICATION
PATENT NO. 0 491 289 A1

Int. Cl.[5]:	G 02 B 21/00
Filing No.:	91121368.4
Filing Date:	December 12, 1991
Priority:	
Date:	December 18, 1990
Country:	DE
No.:	4040441
Publication Date:	June 24, 1992 Patent Bulletin 92/26
Designated Contracting States:	AT BE CH DE DK ES FR GB GR IT LI LU NL SE

DOUBLE-CONFOCAL SCANNING MICROSCOPE

[Doppelkonfokales Rastermikroskop]

Inventor: Dr. Stefan Hell

Applicant: Dr. Stefan Hell

The invention pertains to a scanning microscope with at least one light source, at least /2* one light detector, and at least two objectives that are located on different sides of the object plane and that are arranged so that they can simultaneously illuminate at least one object point, wherein at least one objective is located on a different side of the object plane.

A scanning microscope for transmitted and reflected light is known from DE-OS 3918412, which microscope, as viewed in the direction of the light, has a polarization optical beam splitter in front of and behind a beam scanning device, wherein the rear beam splitter can be switched into and out of the beam path. This known scanning microscope is designed to view samples both with transmitted light and also with reflected light. In contrast to the scanning

* [Ed. note: Numbers in the margin indicate pagination of original document.]

microscope according to the invention, [however, said known microscope] does not improve the resolution by means of interference.

Because the polarization beam splitter leads to different polarization states in the lower and upper beam path, said known scanning microscope is not suitable for creating interference in the focal region of the objective; light of different polarization states is not capable of interference.

In prior microscope developments, special value was placed on the enlargement of the aperture of the microscope objective. This enabled increased resolution in the XY direction. At first, it appeared that a technical limit was reached with an aperture of NA = 0.95 for dry objectives (aperture angle of 71°).

This does not apply to the resolution in the Z direction. However, for the exact three-dimensional detection of a point of transparent or fluorescent objects, the resolution in the Z direction is just as important as in the XY direction.

The task of the present invention is based on disclosing a scanning microscope with a resolution that is as great as possible.

This task is solved according to the invention in that for a scanning microscope of the class mentioned at the beginning of the document, there are interference-modifying means such that light passing through one of the objectives is superimposed with light passing through the objective arranged on the opposite side of the object plane such that an interference pattern is formed at the object and/or at the light detector and an intended change of the interference pattern is possible.

With the interference-modifying means it is possible to influence the resulting interference pattern intentionally so that the interference pattern is not solely dependent on statistical cases or on the arrangement of the microscope parts.

Advantageously, there is at least temporary constructive interference at least at one object point to be imaged and/or at least at one light detector.

Preferably, two objectives that are arranged on different sides of the object plane are aligned opposite each other and centered relative to the optical axis and relative to each other.

According to an especially preferred embodiment, there is at least one aperture in at least one plane that is optically conjugate to the object plane. This aperture is typically a pin diaphragm. An aperture is provided particularly when the plane that is optically conjugate to the object plane is located in front of a light detector that registers the light traveling through the aperture and/or when this aperture acts as a light source.

According to another particularly preferred embodiment, the interference-modifying means is a compensation device that enables coherent or partially coherent wave trains that are completely or partially separated by different objectives to be superimposed at the object. This

compensation device creates at least temporary constructive interference of the wave trains mentioned above at least at one object point to be imaged and/or at least at one light detector. The term compensation device should be understood to mean a device that changes the path difference between wave trains that are passing through or that have passed through different objectives.

Preferably, at least one compensation device as a path-difference modifying element has at least one partially transparent plate with segments of different optical thickness and the plate is moved according to a time sequence such that parts of the plate with different optical thickness are moved into the beam path.

According to another particularly preferred embodiment, at least one compensation device is a mechanical translation device that is attached to at least one optically active part that lengthens or shortens the optical path. A mechanical translation device is, e.g., a piezoelectric or an electromechanical translator. The optically active part can be, in particular, a mirror, a beam or color splitter, or some other deflection element.

In another embodiment, the interference-modifying means preferably generates a translation movement of the objective, so that the translation movement includes a non-disappearing component in the expansion direction of the wavefront, wherein the objective and the sample move together along the optical axis. There can be interference-modifying means /3
of different design in one and the same installation.

Advantageously, the interference-modifying means can change the interference pattern quickly or also slowly. In particular, the compensation device changes the path difference between the wave trains that are passing or that have passed through one objective and the wave trains that are passing or that have passed through the other objective quickly or also slowly. The translation of the objective can likewise be done quickly or also slowly.

According to another particularly preferred embodiment, the interference pattern is changed periodically, the compensation device periodically changes the path difference between the wave trains that are passing or that have passed through one objective and the wave trains that are passing or that have passed through the other objective, or the objective undergoes periodic movements.

Preferably, the function of at least one interference-modifying means is controlled by feedback and/or adjusted with the control and/or regulation electronics of the grid and/or with the image recording electronics.

According to another particularly preferred embodiment, the signal is further processed with the help of sample electronics (lock-in amplifier, boxcar integrator, or the like) and the sample cycle is obtained with the help of an interferometric installation with at least one light detector, or the sample cycle is independent of the optical installation, or the sample cycle is

dependent on signals that are controlled or regulated by the interference-modifying means. In particular, these control and regulation signals include control or regulation signals of mechanical control elements that are attached to the optically active parts and those of compensation devices.

As light detectors, photomultipliers are well-suited, but TV cameras and other receivers that convert light signals into electrical signals can also be used.

Other particularly preferred embodiments are described in the other subordinate claims.

The invention is now explained in more detail with reference to the attached drawings.

Shown are:

Figure 1, the schematic representation of the beam path of a scanning microscope,

Figure 2, the schematic representation of the beam path of another embodiment of a scanning microscope,

Figure 3, the schematic representation of the beam path of another embodiment of a scanning microscope, and

Figure 4, the schematic representation of the beam path of another embodiment of a scanning microscope.

As illustrated in Figure 1, the light source 1 (laser or short-arc lamp) illuminates the pin diaphragm 2. The lens 14 is arranged in front of this pin diaphragm 2 and the lens 3 is arranged behind this pin diaphragm 2 and their distance to the pin diaphragm is preferably their focal length. The wavefront passing through the beam splitter cube 10 is split and deflected by the beam splitter 4 into at least two mutually coherent partial wavefronts. The term wavefront also refers to an entire wave train, i.e., several wavefronts. Here, as in the following, the single wavefront represents a descriptive example for all wavefronts of a wave train. The part of the wavefront split off towards the bottom according to Figure 1 strikes the mirror 6 and is guided from this mirror 6 to the objective 8. The part of the wavefront split off towards the top according to Figure 1 is guided to the mirror 5 arranged preferably symmetric to the mirror 6. This mirror 5 now guides the part of the wavefront incident on this mirror to the objective 7. The two objectives 7 and 8 of the microscope are aligned opposite each other, preferably centered relative to each other and to the common optical axis. The two objectives 7 and 8 focus the partial wavefronts incident on them onto the object plane 9. The object to be examined is located in the object plane 9, so that the common local point, at which the partial wavefronts are focused, in general the common focal point, is the local point to be imaged. The light emitted or reflected from this point is captured by the objectives 7 and 8 and guided by the mirrors 5 and 6 and the beam splitter 4 to the beam splitter cube 10. This beam splitter cube 10 now guides the light or a portion of the light through the lens 11 arranged preferably symmetric to the lens 3 onto the pin

diaphragm 12. The light detector 13 (detector) measures the intensity of the light fed to it through the pin diaphragm 12.

The compensation device 15 is arranged in the beam path between the beam splitter 4 and the mirror 5, and this device changes the optical path difference between the top and bottom partial wavefronts for illumination or detection.

The spatial coherence of the illumination is guaranteed by at least the pin diaphragm 2. In addition, the illumination partial wavefront from above and the illumination partial wavefront /4 from below are capable of interference because they are created from the same light source 1. They interfere within a focal region according to a point-scanning function (PSF) H(x, y, z) that is much more strongly limited spatially than the PSF h(x, y, z) in a conventional (confocal) microscope. If the path difference between the two illumination wavefronts is equal to zero, then the volume of the main maximum of H(x, y, z) is approximately 4 times smaller than the volume of the main maximum of h(x, y, z). This means that the physical-optics resolution limit is clearly reduced. The first intensity minimum of H(x, y, z) along the optical axis lies at approximately one-half wavelength, typically 250 nm, from the absolute intensity maximum at the focal point. In contrast, with h(x, y, z), the first intensity minimum lies more than 1000 nm from the absolute intensity maximum. Analogous to the illumination wavefront, the detection wavefront consists of a partial upper wavefront and a partial lower wavefront. The partial wavefronts are focused in the point detector, where they interfere and generate an imaging procedure according to the point-scanning function H(x, y, z), which is analogous to the illumination (due to reasons of symmetry).

In the double-confocal microscope according to the invention, the quadratic PSF H^2(x, y, z) is responsible for the resolution. The squaring lowers the secondary maximums that result from the interference of the upper and lower wavefronts. The resolution improved in the direction of the optical axis also improves the effective lateral resolution, because the separation of the Z coordinates enables the resolution of lateral characteristics that are otherwise covered up by lateral characteristics of the planes above or below. Because the imaging is done point-wise for scanning microscopes, an improvement of the resolution in the direction of the optical axis is equal to an improvement in the lateral direction. The double-confocal microscope according to the invention exhibits the highest resolution possible for a far-field optical microscope.

If the scanning microscope, whose beam path is illustrated in Figure 1, is used in fluorescence microscopy, then the beam splitter cube 10 is a color beam splitter that allows shorter-wavelength excited light to pass and deflects longer-wavelength light to the side into the light detector 13.

Further, there can be a beam deflection device between the beam splitter cube 10 and the beam splitter 4. The beam splitter 4 that is realized as a mirror in the illustration can be replaced

by other beam splitters (cubes, etc.). Resolution improvement relative to the conventional confocal microscope is also then achieved when the interference only occurs between the upper and lower partial detection wavefronts or when the interference only occurs between the upper and lower illumination wavefronts.

In the case of fluorescence microscopy, in the wavefronts deflected upwards or downwards by the beam splitter 4, one of the illumination or detection beam paths can be stopped with the help of color filters. This lowers the resolution, but the resolution is still greater than for conventional confocal microscopes.

The compensation device 15, which is, e.g., an optical delay plate, is installed for optimum adjustment of the interference, particularly so that there is constructive interference. The device can be installed either as shown in the upper part of the wavefront or also in the lower part of the wavefront. In particular, the compensation device can adjust the interference and its delay can be quickly changed, temporarily. The device can be controlled by feedback with the image recording electronics and/or the electronics for control and regulation of the grid.

If object scanning is performed, the object is located on a scan table, not indicated here, which enables the translation of the object as much as possible in the X, Y, and Z directions. If beam scanning is performed, a suitable scan table preferably moves along the optical axis (Z axis).

Figure 2 shows the schematic representation of another embodiment of a scanning microscope that is particularly suited for fluorescence microscopy, wherein parts that are equal to the parts shown in Figure 1 are provided with the same reference symbols.

The light from the light source 1 is guided through the beam splitter 10 to the (geometric) beam splitter 4 that splits the wavefront into two interference-capable portions. One part is guided by means of deflection elements into the upper objective 7 of the microscope (upper illumination wavefront) and the other part is guided by means of deflection elements into the lower objective 8 of the microscope (lower illumination wavefront). Both illumination wavefronts interfere in the common object plane 9. Portions of the light output from there are collected by the objectives 7 and 8. The light collected by the objective 7 (upper detection wavefront) and likewise the light collected by the objective 8 (lower detection wavefront) are guided by means of deflection elements to the beam splitter 4, which combines the two detection wavefronts. The detection wavefronts are guided by means of the beam splitter 10 to the pin diaphragm 12. The light intensity fed through the pin diaphragm 12 is measured by a light detector 13 and serves as a signal, i.e., as a characteristic of the object point to be imaged. /5

Due to the design according to the invention, there is interference of the upper and lower detection or illumination wavefronts in at least one of the planes conjugate to the object plane 9 or in the object plane 9 itself.

In order to guarantee the interference, be able to perform controlled interference, or to guarantee constructive interference, the compensation devices 27 and 28 for changing the path difference between the upper and lower wavefronts are arranged in the beam path between the beam splitter 4 and the objective 7. It is also possible to arrange these compensation devices in the beam path between the beam splitter 4 and the objective 8. Optionally, compensation can also be performed by the movement of certain components, particularly the deflection elements described in the following. The compensation device or the optical paths are dimensioned so that in each case there is a higher degree of coherence between the upper and lower wavefronts. Therefore, there can be interference in every case.

As can be seen from Figure 2, the beam for this embodiment is optionally enlarged or reduced in its diameter with the help of the components 60 and 60' that are arranged between the beam splitter cube 10 and the beam splitter 4. Furthermore, the beam passes through the beam deflection unit 50. As already described above, the wavefront input to the beam splitter 4 is divided into an upper and a lower illumination wavefront. In the case of fluorescence microscopy according to Figure 2, the beam splitter cube 10 is a color splitter cube that deflects the light to be detected into the light detector and that feeds the illumination light to the beam splitter. Between the beam splitter 4 and the objective 7 and/or between the beam splitter 4 and the microscope objective 8, there is at least one color splitter. According to Figure 2, the two color splitters 20 and 21 separate the detection wavefront from the illumination wavefront. These separated wavefronts are guided to a compensation device 27 and 28, respectively, so that the illumination and detection wavefronts can be changed in phase and/or amplitude separately.

For the case that the detected light has a longer wavelength and the color splitter 20 and 21 reflect longer-wavelength light and transmit shorter-wavelength light, the compensation device 27 performs the compensation of the illumination wavefront and the compensation device 28 performs the compensation of the detection wavefront. The term compensation is to be understood to mean the change of the path difference between the partial wavefronts and/or the change of the phase of a wavefront.

Analogous to the already described color splitters 20 and 21, there are the color splitters 22 and 23 in the lower beam path. These color splitters 22 and 23 are designed analogous to the upper beam path. Preferably, they can be removed from the beam path and/or replaced with color splitters with other physical properties. Additional color splitters or color splitter pairs can be installed between the beam splitter 4 and the objective 7 analogous to the color splitters 20 and 21, and also between the beam splitter 4 and the objective 8, e.g., for multiple fluorescence, in order to be able to also change wavefronts with other wavelengths in the path difference of the partial wavefronts.

By means of the deflection element 5', the upper illumination wavefront is guided to the compensation device 27 that changes the optical path length or the phase of the wavefront. By means of the deflection element 5, the upper illumination wavefront is guided into the objective 7 which focuses the light in the object plane 9. By means of the deflection element 6' and the deflection element 6, the lower illumination wavefront is guided into the objective 8 which likewise focuses the light at the common focal point. At the focal point, the two illumination wavefronts can form interference relative to each other.

The object is preferably located on a table device that enables the object to be moved in the Z direction, preferably in all three spatial directions.

The light output from the object point is guided into the objectives 7 and 8, which form the upper and lower detection wavefronts, respectively. In general, the term "wavefront" also refers to other wavefronts of the same wave train. For fluorescence operation, the upper detection wavefront is guided by means of the color splitter 21, the compensation device 28, and the color splitter 20 to the beam splitter 4, and likewise, the lower detection wavefront is guided by the color splitters 23 and 22 to the beam splitter 4. In non-fluorescence operation, the detection wavefronts pass through the color splitters and are deflected by the deflection elements 5 and 5' or 6 and 6' to the beam splitter 4. The beam splitter 4 combines the two detection wavefronts into one. The beam splitter 10 guides the detection wavefront to the lens 11. The detection wavefront is converted into a spherical wavefront that converges in the pin diaphragm 12. The two detection wavefronts interfere in the plane of the pin diaphragm 12 with each other and image the object point of the object plane 9 in the plane of the pin diaphragm 12 with an enlarged aperture in the sense of the double confocal microscope. /6

In the sense of the invention, this arrangement forms interference of the partial illumination or detection wavefronts at the object and/or at the light detector. Therefore, it is possible to not use a detection wavefront or an illumination wavefront.

For this purpose, either the upper or the lower illumination wavefront is interrupted. This is done with the help of opaque obstacles 45 and 46 in the upper and lower illumination wavefronts, respectively. With the help of opaque obstacles 47 and 48 in the upper and lower detection wavefronts, respectively, the upper or lower detection wavefront is stopped. Here, a one-sided detection and illumination with interfering illumination wavefronts is possible.

For the embodiment described in Figure 2, the beam deflection unit is designed so that the beam is moved by an angle relative to the optical axis, wherein the centers of the entrance pupils 7' and 8' of the objectives 7 and 8, respectively, are preferably points of rotation. Deflection units that can be adjusted relative to each other can also be inserted between the beam splitter 4 and the objective 7 or between the beam splitter 4 and the objective 8.

The changing of the relative path difference of the upper and lower wavefronts can also be done by quickly changing the dimensions of the upper and lower beam paths, e.g., by means of simultaneously moving deflection units 5 and 5' or the color splitter pairs 20 and 21 in the direction of the optical axis. This motion can be done, e.g., with the help of piezoelectric or electromechanical control elements.

The frequency for changing the path difference by the compensation device can be detected with the help of an interferometric arrangement. The signal can be used as a clock generator for the sample electronics (lock-in amplifier, boxcar integrator).

The schematic representation of the beam path of another embodiment of a scanning microscope is illustrated in Figure 3, wherein parts corresponding to parts illustrated in Figures 1 and 2 are provided with the same reference symbols. In the following, according to Figure 3, only the parts of the beam path, which differ from the already described beam paths, will be explained in more detail.

There is a beam deflection unit between the beam splitter 10 and the beam splitter 4. The wavefront output from the light source 1 through the pin diaphragm 2, the lens 3, and then the beam splitter 10 is guided to the mirror 51, which can be quickly tilted about an axis (e.g., perpendicular to the plane of reflection). Usually the mirror is connected to the axis of a d'Arsonval galvanometer, which is operated with the help of a sinusoid voltage. In this way the beam is tilted in the plane of reflection (= plane of the paper) according to the mirror 51. The lenses 52 and 53 are arranged so that the mirror 51 images into the mirror 54. The mirror 54 undergoes a tilting motion that is perpendicular to the mirror 51. The lenses 55 and 56 image the mirror 54 and thus also the mirror 51 into the entrance pupils 7' and 8' of the objectives 7 and 8. The beam undergoes a rotating motion in two directions that are perpendicular to each other in the entrance pupils 7' and 8'. The points of rotation are located in the entrance pupils 7' and 8'. The entrance pupils 7' and 8' of the mirrors 54 and 51 are located in planes that are optically conjugate relative to each other.

The motions of the mirrors 51 and 54 are converted by the two objectives 7 and 8 of the microscope in the object plane 9 into two linear motions perpendicular to each other. In this way, the object can be scanned over a surface. If one of the two mirrors is connected to a two-axes mechanism, e.g., if one galvanometer mirror is placed on the other, then one mirror and two lenses can be eliminated. This is good for the yield of the detected light. One mirror can also be eliminated, if a deflection direction is replaced by an equivalent table motion in the object plane 9.

In order to guarantee interference, be able to guarantee controlled interference, or to guarantee constructive interference, there is at least one compensation device 27 or 28 for changing the path difference between the upper and lower wavefronts in the beam path between

the beam splitter 4 and the objective 8. Optionally, the compensation can also be performed by moving certain components, particularly the deflection elements 25 and 26. The compensation device or the optical paths are dimensioned so that there is a high degree of coherence between the upper and lower wavefronts in order to enable interference. In Figure 3, the other compensation device 29 is arranged in the beam path between the beam splitter 4 and the objective 7.

An essential difference between the confocal and the double-confocal microscope described here consists in the fact that for the double-confocal microscope, either the illumination wavefront consists of parts that are incident on the optical axis from opposite directions and that interfere with each other, or the detection wavefront consists of parts that initially move apart in opposite directions of the optical axis from the object and that interfere with each other at the light detector, or they are both incident simultaneously. /7

In Figure 4, the beam path of another embodiment of a scanning microscope is shown schematically. This arrangement differs from the previously described embodiment of a scanning microscope in that the wavefronts are separated into an upper and a lower illumination wavefront before the pin diaphragm. Due to these reasons, there are two pin diaphragms 102 and 202, which are responsible for the upper and lower wavefront, respectively. After first passing through the pin diaphragms 102 and 202, the detection wavefronts are then combined. As before, the illumination wavefronts interfere in the object and the detection wavefronts interfere in the detector 13.

Another difference between the previously described arrangements is that the two pin diaphragms 102 and 202 can perform translation motions and can be displaced relative to each other.

The remaining components satisfy the same functions as with the other arrangements.

The shifting of the pin diaphragm 102 is preferably performed together with the lens 101 and/or the mirror 5; the shifting of the pin diaphragm 202 is performed analogously with the lens 201 and/or the mirror 6. The shifting of the pin diaphragms 102 and 202 produces an intended change in the interference pattern and thus a somewhat different type of imaging.

For the other arrangements, all of the beam splitters can also be geometric (e.g., corner mirror) or physical wavefront splitters (e.g., beam splitter cube), or they can consist of several, optionally also individual optically active parts. In the case of fluorescence microscopy, the beam splitter 110 and/or 210 can be a color splitter.

The beam splitter 4 divides the wavefront output from the laser into an upper and a lower illumination wavefront. The prism 4' deflects the lower illumination wavefront to the deflection element 6'. From there, this is guided by the deflection element 6 and the beam splitter cube 210 to the lens 201. The upper illumination wavefront is guided by the compensation device 27

through the deflection element 5' and 5 and then through the beam splitter cube 110 to the lens 101. The upper detection wavefront is guided by the pin diaphragm 102 and the lens 101 to the beam splitter 110, where it is guided by the deflection element 105 (e.g., mirror or prism) to the compensation device 28, and from there it is guided to the beam splitter 304, where it is united with the lower detection wavefront. The lower detection wavefront is guided by the beam splitter 210 and the deflection element 206 to the beam splitter 304. The united wavefront is guided by the lens 11 to the pin diaphragm 12.

In this arrangement, as in all the other arrangements, the beam splitter 4 can be both a physical and also a geometric beam splitter, or it can also be assembled from several optically active parts. This also applies for the beam splitter 304, which can also by replaced by a combination, like that formed by 4 and 4'.

Claims

1. Scanning microscope with at least one light source (1), at least one light detector (13), and at least two objectives (7, 8), wherein at least one of the objectives (7, 8) located on different sides of the object plane (9) is directed towards at least one objective located on the other side of the object plane, characterized in that there is at least one interference-modifying means such that light passing through at least one of the objectives (7) is superimposed coherently or semi-coherently on light passing through at least one objective (8) located on the other side of the object plane (9), so that the superimposed light waves at the object and/or at least at a light detector (13) at least temporarily interfere with each other, in order to realize an intended change of the interference.

2. Scanning microscope according to Claim 1, characterized in that there is at least temporary constructive interference at least at one object point to be imaged and/or at least at one light detector.

3. Scanning microscope according to Claim 1 or 2, characterized in that two objectives (7, 8) arranged on different sides of the object plane (9) are directed towards each other and centered relative to the optical axis and relative to each other.

4. Scanning microscope according to Claim 1, 2, or 3, characterized in that there is at least one aperture (12, 2) in at least one plane that is optically conjugate to the object plane. /8

5. Scanning microscope according to at least one of the preceding claims, characterized in that the interference-modifying means is a compensation device (15, 27, 28, 29) that enables at least at one object point to be imaged and/or at least at one light detector a superposition of coherent or partially coherent wave trains that each pass through different objectives (7, 8) entirely or partially.

6. Scanning microscope according to Claim 5, characterized in that the compensation device (15, 27, 28, 29) creates at least temporary constructive interference of the wave trains at least at one object point to be imaged and/or at least at one light detector (13).

7. Scanning microscope according to Claim 5 or 6, characterized in that at least one compensation device (15, 27, 28, 29) has, as a path-difference modifying element, an at least partially transparent plate that has segments of different optical thickness and the plate is moved so that according to a time sequence parts of the plate with different optical thickness are moved into the beam path.

8. Scanning microscope according to Claim 5 or the following, characterized in that at least one compensation device is a mechanical translation device that is connected to at least one optically active part to lengthen or shorten the optical path.

9. Scanning microscope according to at least one of the preceding claims, characterized in that at least one interference-modifying means produces a translation motion of the objectives, so that the translation motion has a non-disappearing component in the expansion direction of the wavefront passing through it, wherein the objectives and the sample move together.

10. Scanning microscope according to at least one of the preceding claims, characterized in that the interference-modifying means can change the interference quickly or also slowly.

11. Scanning microscope according to at least one of the preceding claims, characterized in that the changing of the interference is performed periodically, that the compensation device periodically changes the path difference between the wave trains that are passing through or that have passed through one objective and the wave trains that are passing through or that have passed through the other objective, or that the objectives undergo a periodic motion.

12. Scanning microscope according to at least one of the preceding claims, characterized in that the function of at least one interference-modifying means is controlled by feedback and/or adjusted with the control and/or regulation electronics of the scanning and/or with the signal processing electronics.

13. Scanning microscope according to at least one of the preceding claims, characterized in that the signal is further processed with the help of sample electronics (lock-in amplifier, boxcar integrator, or the like) and that the sample cycle is obtained with the help of an interferometric design with at least one light detector, or that the sample cycle is independent of optical design, or that the sample cycle is relative to signals that are controlled or regulated by the interference-modifying means.

14. Scanning microscope according to at least one of the preceding claims, characterized in that the signal evaluation is done at least partially with an electronic data processing machine (computer).

15. Scanning microscope according to at least one of the preceding claims, characterized in that there is at least one device for changing the amplitude in the illumination and/or the detection beam paths.

16. Scanning microscope according to at least one of the preceding claims, characterized in that there is at least one device that at least partially compensates for wavefront aberrations /9
resulting particularly from cover glass or samples.

17. Scanning microscope according to at least one of the preceding claims, characterized in that the apertures that are optically conjugate relative to the object plane can be removed and/or exchanged and/or varied in their opening and/or moved, i.e., by translation and/or rotation.

18. Scanning microscope according to at least one of the preceding claims, characterized in that there are additional optical elements with at least one light detector, particularly in order to produce an image with conventional or typical confocal resolution in addition to the double-confocal image.